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1083-8791/06/1204-0008$32.00/0
doi:10.1016/j.bbmt.2005.12.030

Unrelated Donor Granulocyte Colony-Stimulating Factor–Mobilized Peripheral Blood Mononuclear Cell Transplantation after Nonmyeloablative Conditioning: The Effect of Postgrafting Mycophenolate Mofetil Dosing

Michael B. Maris,[1,2] Brenda M. Sandmaier,[1,2] Barry E. Storer,[1,2] David G. Maloney,[1,2] Judith A. Shizuru,[3] Edward Agura,[4] Constanze Kliem,[5] Michael Pulsipher,[6] Richard T. Maziarz,[7] Peter A. McSweeney,[8] James Wade,[9] Amelia A. Langston,[10] Thomas R. Chauncey,[1,2,11] Benedetto Bruno,[12] Karl G. Blume,[3] Rainer Storb[1,2]

[1]Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]University of Washington, Seattle, Washington; [3]Stanford University, Palo Alto, California; [4]Baylor University, Dallas, Texas; [5]University of Leipzig, Leipzig, Germany; [6]University of Utah, Salt Lake City, Utah; [7]Oregon Health and Science University, Portland, Oregon; [8]University of Colorado, Denver, Colorado; [9]Medical College of Wisconsin, Milwaukee, Wisconsin; [10]Emory University, Atlanta, Georgia; [11]Seattle Veterans Administration Medical Center, Seattle, Washington; and [12]University of Turin, Turin, Italy

Correspondence and reprint requests: Rainer Storb, MD, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., D1-100, P.O. Box 19024, Seattle, WA 98109-1024 (e-mail: rstorb@fhcrc.org).

Received September 26, 2005; accepted December 5, 2005

ABSTRACT

We previously reported results in 71 patients with advanced hematologic malignancies given HLA-matched unrelated granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cell (G-PBMC) grafts after fludarabine 90 mg/m^2, 2 Gy of total body irradiation, and postgrafting mycophenolate mofetil (MMF) 15 mg/kg twice daily and cyclosporine 6.25 mg/kg twice daily orally. Graft rejection was 15%; the cumulative probability of acute graft-versus-host disease (GVHD) was 52%. According to MMF pharmacokinetic studies, which showed a short half-life of its active metabolite, mycophenolic acid, we increased MMF dosing from 15 mg/kg twice daily to 15 mg/kg 3 times daily to increase immunosuppression and reduce the incidence of both graft rejection and acute GVHD. Among 103 patients so treated, graft rejection occurred in 5%, whereas acute GVHD remained at 53%. Outcomes were compared with results of previous G-PBMC recipients given MMF twice daily. Infection rates were slightly higher with MMF 3 times daily than with MMF twice daily. Nevertheless, 2-year nonrelapse mortality and overall and progression-free survivals were similar for MMF 3-times-daily and twice-daily patients (19%, 58%, and 49% versus 20%, 48%, and 37%, respectively). Nonmyeloablative conditioning with postgrafting cyclosporine and MMF given 3 times daily allowed 95% durable engraftment of unrelated donor G-PBMC grafts.
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KEY WORDS

Hematopoietic cell transplantation • Nonmyeloablative conditioning • Reduced-intensity conditioning • Unrelated donor allografting • Hematologic malignancy • Graft-versus-tumor effect

INTRODUCTION

In 2003, we reported initial results in 71 patients with advanced hematologic malignancies who received HLA-matched unrelated granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cell (G-PBMC; 15%) grafts using a nonablative conditioning regimen that combined 3 doses of fludarabine (FLU) and 2 Gy of total body irradiation (TBI) [1]. Postgrafting immunosuppression, aimed both at facilitating allogeneic hematopoietic engraftment and controlling graft-versus-host disease (GVHD), consisted of twice-daily mycophenolate mofetil (MMF) for 3 months and twice-

daily cyclosporine (CSP) for 6 months. The nonablative regimen was chosen because patients were deemed too old and/or too ill to tolerate conventional high-dose hematopoietic cell transplantation (HCT). With follow-ups ranging from 0.5 to 2.4 years, the overall survival of the heterogenous group of patients was 57%, and clear evidence of graft-versus-tumor (GVT) effects was observed. Even so, 43% of patients relapsed or progressed, and 27% died from their underlying diseases. Some, although not all, of the relapses were the consequence of a 15% rate of graft rejection, which was followed by autologous hematopoietic recovery in all but 2 patients. Other causes of failure included complications from acute GVHD, which was seen in 52% of patients.

This study included 103 patients with malignancies given unrelated HCT and was designed to address both graft rejection and acute GVHD by introducing 1 change to the original protocol: increasing MMF dosing from 2 to 3 times daily. This change was prompted both by observations on the importance of MMF for engraftment, originally made in preclinical canine studies [2], and by the short (3-hour) half-life of mycophenolic acid, the active metabolite of MMF, in the prior clinical study [1]. We hypothesized that more frequent MMF dosing would overcome the disadvantage of the short mycophenolate acid half-life and provide improved immunosuppression.

This article describes the results in the 103 new patients and then compares the observed incidences of graft rejection and acute GVHD with those among the 71 previously reported G-PBMC recipients. Finally, the combined data of all 174 G-PBMC recipients from both studies are analyzed for outcomes.

MATERIALS AND METHODS

Eligibility Criteria

Patients in this study were treated at 11 academic centers including the Fred Hutchinson Cancer Research Center (FHCRC), University of Washington Medical Center, Seattle Children's Hospital and Regional Medical Center, Seattle Veterans Administration Medical Center, Stanford University, Baylor University, University of Leipzig (Germany), University of Utah, Oregon Health and Science University, University of Colorado, Emory University, Medical College of Wisconsin, and the University of Turin (Italy): the FHCRC acted as the coordinating center. The institutional review board at each center approved FHCRC protocol 1641. Informed written consent was obtained from all patients. All patients older than 50 years were eligible for the study (n = 70). Patients younger than 50 years were included if they had comorbid conditions that excluded them from conventional allogeneic HCT. Conditions included failed previous high-dose autologous HCT (n = 18), recent pneumonitis (n = 1), liver dysfunction (n = 2), cardiomyopathy (n = 1), dyskeratosis congenita (n = 1), renal dysfunction (n = 1), metastatic renal cell carcinoma (n = 4), morbid obesity (n = 1), and morbidity from multiple prior chemotherapies (n = 2). In 3 instances, inclusion in the protocol was due to patient preference.

Excluded from the protocol were patients who were pregnant or who had rapidly progressive B- or T-cell malignancies, myeloid malignancies with >5% marrow blasts [1], a Karnofsky performance status of <50%, decompensated liver disease, a corrected pulmonary diffusion capacity of <40%, a cardiac ejection fraction of <35%, or serologic evidence of infection with the human immunodeficiency virus. No exclusions were made for renal insufficiency or active bacterial or fungal infections, although fungal pneumonitis needed to be stable or improved after 1 month of azole therapy.

HLA Typing and Matching

One hundred three donors and recipients were selected on the basis of matching by intermediate- and high-resolution typing for HLA-A, -B, and -C and high-resolution typing for -DRB1 and -DQB1 [3]. Ninety-three pairs were matched at the allele level for both HLA class I and II antigens. Ten pairs were mismatched: 6 for 1 HLA-C allele, 3 for 1 HLA-B allele, and 1 for an HLA-A allele each.

Patient Characteristics

One hundred four sequential patients were enrolled on FHCRC protocol 1641 between October 2002 and August 2003. One patient withdrew consent during FLU conditioning and did not receive TBI or the infusion of unrelated G-PBMC. Only data from the 103 patients who received HCT are presented. Patient characteristics are shown in Table 1. The median patient age was 54 years (range, 18-69.6 years). The patients' diagnoses included myelodysplasia (MDS; n = 9), acute myeloid leukemia (n = 23), acute lymphocytic leukemia (n = 1), chronic myeloid leukemia (CML; n = 5), non-Hodgkin lymphoma (NHL; n = 24), myeloproliferative syndrome (MPS; n = 3), chronic lymphocytic leukemia (CLL; n = 13), Hodgkin disease (HD; n = 8), multiple myeloma (n = 11), renal cell carcinoma (n = 4), and Waldenström macroglobulinemia (n = 2). The median time from diagnosis to transplantation was 27 months (range, 3-191 months).

Conditioning Regimen and Postgrafting Immunosuppression

FLU was given intravenously at 30 mg/m^2/d on days −4, −3, and −2 before HCT, and 2 Gy of TBI was delivered at 0.07 Gy/min from a linear accelerator

Table 1. *Patient and Disease Characteristics*

Variable	3-Times–Daily MMF	Twice-Daily MMF	Overall
No. patients	103	71	174
Median age, y (range)	54 (17-69.6)	54 (18-70)	54 (17-70)
Median No. of preceding therapies (range)	4 (0-12)	3 (0-11)	3 (0-12)
Median follow-up after HCT, mo (range)	21.4 (6-34.7)	38.7 (18.3-51.6)	24.9 (6-51.6)
Preceding HCT	37%	30%	34%
Median No. CD34 cells × 10^6/kg (range)	7.3 (0.8-26.3)	7.0 (1.2-24.4)	7.2 (0.8-26.3)
Median No. CD3 T cells × 10^8/kg (range)	2.7 (0.3-9.3)	2.6 (0.8-5.8)	2.6 (0.3-9.3)
Donor HLA match (No. patients)			
HLA allele match	93	58	151
HLA allele mismatch	10	13	23
Diagnosis and status at HCT (No. patients)			
AML/ALL (total)	24	13	37
CR1	8	6	14
CR2	13	2	15
>CR3	1	4	5
Rel/Ref/induction failure	2	1	3
MDS (total)	9	17	26
RA	2	3	5
RAEB/CR1	1/3	2/0	3/3
RAEB-T	0	3	3
AML CR1/CR2	3/0	2/1	5/1
AML Rel/Ref	0/0	2/1	2/1
CMML PR/Ref/induction failure	0/0/0	1/1/1	1/1/1
CML (total)	5	10	15
CP/AP/BC	3/2/0	6/1/0	9/3/0
CP-2	0	3	3
MPS (total)	3	5	7
ET	0	2	2
AMM/transformation	1/0	0/1	1/1
AML CR	1	0	1
NOS	1	2	3
Indolent/high-grade NHL (total)	24	10	34
CR/PR	9/6	2/2	11/8
Stable	1	0	1
Ref/Rel	6/2	4/2	10/4
CLL (total)	13	5	18
FLU sensitive	0	1	1
FLU refractory	13	4	17
HD (total)	8	4	12
CR/PR	1/3	0/2	1/5
Rel/Ref	1/3	0/2	1/5
MM (total)	11	7	18
CR/PR/Ref	2/6/3	0/4/3	2/10/6
Waldenström	2	0	2
Renal cell carcinoma	4	0	4
High risk diagnoses*	90 (87%)	54 (76%)	144 (83%)

ALL indicates acute lymphocytic leukemia; AML, acute myeloid leukemia; AMM, transformation-agnogeneic myeloid metaplasia in transformation; AP, accelerated phase; CLL, chronic lymphoid leukemia; CML, chronic myelogenous leukemia; CP, chronic phase; CR, complete remission; ET, essential thrombocytosis; HD, Hodgkin disease; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPS, myeloproliferative syndrome; NHL, non-Hodgkin lymphoma; NOS, not otherwise specified; PR, partial remission; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation; Ref, refractory; Rel, relapsed; BC, blast crisis; CMML, chronic myelomonocytic leukemia.

*High-risk malignancies include advanced-stage AML, ALL, MDS, CML in accelerated phase or blast crisis, or B-cell malignancies.

on day 0. Immunosuppressive therapy with oral CSP 6.25 mg/kg twice a day was started on day −3, and MMF 15 mg/kg orally 3 times a day was started 4 to 6 hours after HCT on day 0. CSP levels were measured by immunoassay and were targeted to 400 to 500 ng/mL for the first month and 300 ng/mL thereafter. CSP and MMF were administered intravenously if patients were not able to tolerate oral medications. For patients without manifestations of GVHD, MMF was tapered at day 40 over 56 days, and CSP was tapered at day 100 over 80 days.

Collection of Hematopoietic Cells

Donor G-PBMC collections were conducted by National Marrow Donor Program standards. Red blood

cell (RBC) depletions of grafts were performed for patients with high titers of antidonor isohemagglutinins. A portion of the G-PBMC product, containing at least $1 \times 10^7 CD3^+$ cells per kilogram, was cryopreserved for potential use as donor lymphocyte infusions (DLIs).

Supportive Care

At most collaborating institutions, patients were seen in the ambulatory care setting except during the scheduled infusion of the hematopoietic cells and whenever transplantation complications mandated closer medical supervision or therapy. All patients at the University of Leipzig were treated for at least 1 month on a hematology ward per institutional practice. Supportive care measures have been previously described [1].

GVHD Grading and Therapy

The GVHD grade was assigned by local investigators by using standard criteria [4-6]. Treatment of acute GVHD was conducted according to each institution's standard practice guidelines.

Treatment of Persistent/Progressive or Relapsed Malignancies

Progressive or relapsed malignancies in the absence of severe manifestations of acute or chronic GVHD were treated by rapid discontinuation of systemic immunosuppression to initiate GVT effects [7,8]. In addition, 5 patients received DLI for disease progression (n = 3) [9], low (<40%) CD3 chimerism (n = 1) [10], or Epstein-Barr virus lymphoproliferative disease in donor B cells (n = 1).

Chimerism Analyses

Nucleated cells from the marrow and T cells and granulocytes from the peripheral blood were isolated by using flow cytometry on days 28, 56, 84, 180, 365, and 545 and then yearly after HCT for chimerism analyses. Percentages of donor-host cell chimerism levels for recipients of sex-mismatched HCT were evaluated by fluorescent in situ hybridization for X and Y chromosomes [11], whereas those for recipients of sex-matched HCT were determined by polymerase chain reaction–based amplification of variable number tandem repeat sequences unique to donors and hosts [12]. Fluorescent in situ hybridization tests were performed, whenever possible, because of their slightly better sensitivity and their faster turnaround time, the latter being important in cases of suspected graft rejection. Donor-host chimerism was assessed exclusively by variable number tandem repeat for all patients treated at Stanford University. Chimerism was determined by visual inspection of silver-stained agarose gels with a standard error of 1% to 5%.

Study End Points

Data were analyzed as of December 1, 2004. The 2 primary objectives of this protocol were to (1) reduce the risk of true graft rejection in patients without preceding chemotherapy to <20% and in those with preceding chemotherapy to <10% and (2) reduce the risk of grade II to IV acute GVHD in patients with sustained engraftment to <35%. Secondary end points included survival and progression-free survival.

Comparison of Results in Current and Previously Published G-PBMC Recipients

Results in the previously published 71 G-PBMC recipients on FHCRC protocol 1463 (MMF twice daily) were updated as of December 2004. Their characteristics are also shown in Table 1. Graft rejection and acute GVHD incidences in patients on both the current protocol (1641) and the previous protocol (1463) were compared, and then data from both patient groups were combined for multivariate outcome analyses.

Statistical Methods

The percentages of patients with sustained engraftment (based on detection of >5% donor CD3 chimerism) were compared by the χ^2 test. Overall and progression-free survivals were estimated by the Kaplan-Meier method. Cumulative incidence estimates were calculated for graft rejection, acute GVHD, relapse, and nonrelapse mortality. Hazard ratios were estimated from Cox regression models. All *P* values were derived from likelihood ratio statistics and were 2 sided. Patients were considered to have died of nonrelapse causes if there was no evidence of disease relapse or progression. Patients with persistent disease were considered at risk for nonrelapse mortality. Disease responses and progression were defined by standard criteria. Neutrophil recovery was defined as the first of 3 consecutive days with neutrophil counts $>0.5 \times 10^9$/L. Platelet recovery was defined as the first of 3 consecutive days with platelet counts $>20 \times 10^9$/L without transfusion support.

Outcome Analysis of Combined Current and Previous Data

For the evaluation of global risk factors for rejection, low chimerism, relapse/progression, and overall and progression-free survival, updated data from 71 G-PBMC recipients given MMF twice daily were combined with the data from the 103 patients given MMF 3 times daily. Multivariate models were constructed in a stepwise fashion by using a threshold significance level of .05 for inclusion in the model. Multivariate *P* values for a variable were based on adjustment for all other variables in the model. Pretransplantation factors considered in this analysis in-

cluded diagnosis, disease type, CD34 dose, CD3 dose, patient sex, donor sex, sex mismatch, HLA class I allele mismatch, preceding transfusions, preceding chemotherapy, and preceding autologous transplantation. Of note, 2 additional patients in the previous cohort experienced late graft rejections (400 and 1123 days after HCT, respectively), thus bringing the overall graft rejection rate to 18% (13/71).

RESULTS

Engraftment

The median CD34 and CD3 cell doses of G-PBMC were 7.3×10^6 (range, 0.8-26.3 $\times$ 10^6) and 2.7×10^8 (range, 0.3-9.3 $\times$ 10^8) per kilogram recipient body weight, respectively (Table 1). One of 103 patients studied did not recover neutrophil counts to $>0.5 \times 10^9$/L (early graft rejection), and a second patient had a loss of neutrophil production after initial recovery (late graft failure along with decreasing donor CD3 chimerism). This patient was then given previously stored autologous G-PBMC but died of acute respiratory distress syndrome before recovery of autologous cells. Eighteen (16%) of 103 patients did not develop neutropenia ($<0.5 \times 10^3/\mu L$). The median neutrophil nadir was 100 cells per microliter, and the duration of neutropenia was 7 days (range, 0-44 days). Sixty-six (64%) of the 103 patients did not develop thrombocytopenia ($<20 \times 10^3/\mu L$). The median platelet nadir was 32 000/μL, and the median time to platelet recovery $>20 \times 10^3/\mu L$ was 0 days (range, 0-27 days). The median numbers of RBC and platelet transfusions were 6 (range, 0-32) and 2 (range, 0-48), respectively. RBC and platelet transfusions were not required in 21 (20%) and 60 (58%) of the 103 patients, respectively.

Initial donor engraftment at day 28 (defined as >5% donor T-cell chimerism) was observed in 101 (98%) of the 103 patients and was sustained in 98 patients (95%). The sustained engraftment rate was higher for patients with preceding chemotherapy (88 of 91; 97%) than in those without such therapy (10 of 12; 83%; P = .05). The 97% engraftment rate for the 88 patients with preceding chemotherapy met the 95th percentile confidence intervals set forth in the protocol objectives for achieving <10% rejection (P = .05). However, there was insufficient statistical power to demonstrate that the 17% rejection rate among the 12 patients without preceding chemotherapy truly resulted in a rejection rate of <20% (P = .33). Overall, 5 patients with acute myeloid leukemia, mantle cell lymphoma, multiple myeloma, and CML in chronic phase (CP; n = 2) experienced graft rejection (2 complicated by aplasia). Three of these patients had successful engraftment after 1 (n = 1) and 2 (n = 2) further unrelated donor HCT attempts with FLU 90 mg/m^2 and 3 to 4 Gy of TBI or cyclophosphamide 120 mg/kg, FLU 150 mg/m^2, and Thymoglobulin 7.5 mg/kg. Unfortunately, 1 of these patients died of sepsis. The patients with CML-CP refused to consider a second transplantation. Most patients had high degrees of donor chimerism levels in nucleated marrow cells and peripheral blood granulocytes and T cells at day 28, with gradual increases over the subsequent 730 days (Figure 1A).

Toxicities and Nonrelapse Mortality

Twenty of the 103 patients were not hospitalized. Eighty-three (81%) required a median of 11 days (range, 0-100 days) of hospitalization. Alopecia, mucositis, and veno-occlusive disease were not observed. The numbers of grade III and IV organ toxicities within the first 100 days are shown in Table 2. Fifty-six (54%) patients experienced 107 grade III events, and 17 patients experienced 20 grade IV toxicity events.

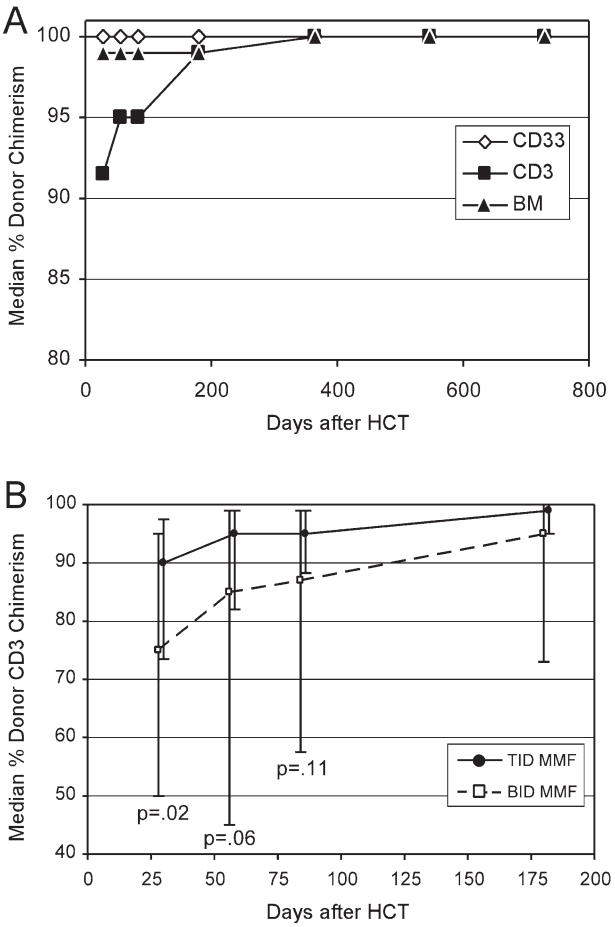

Figure 1. A, Median percentages of granulocyte (CD33), T-cell (CD3$^+$), and whole nucleated marrow cell (BM) chimerism levels after HCT in 103 patients given G-PBMC grafts and MMF every 8 hours. B, Median percentages of donor T-cell chimerism levels for patients given MMF every 8 (TID) or 12 (BID) hours a day. The bars in (B) indicate the 95th percentile.

Table 2. *Numbers of Grade III and IV Nonhematopoeitic Toxicities within 100 Days of HCT (n = 103)*

Organ System	Event	No. Events*	
		Toxicity Grade III	Toxicity Grade IV
Cardiovascular	Hypertension	1	0
	Hypotension	4	2
	Atrial dysrhythmia	3	0
	Chest pain	1	0
	Infarction	0	1
	Congestive heart failure	3	0
	Deep venous thrombosis	3	0
	Cardiac arrest	0	3
Endocrine	Syndrome of inapropriate antidiuretic hormone	1	1
Gastrointestinal	Nausea and vomiting	5	1
	Diarrhea	2	0
	Constipation	1	0
	Stomatitis	1	0
Hemorrhage	Hematuria	3	0
	Conjunctival	1	0
	Right groin (procedure related)	1	0
	Gastrointestinal	1	0
	Subdural hematoma	0	1
Hepatic	Hyperbilirubinemia	29	0
	Elevated transaminases	4	0
	Hypoalbuminemia	1	0
	Lactate dehydrogenase elevation	1	0
Metabolic	Hyperglycemia	1	0
	Electrolyte abnormality	1	0
Neurologic	Cerebral vascular accident/infarct	0	2
	Mental status change	1	1
	Syncope	0	0
	Depression	1	0
	Weakness	2	0
	Insomnia	1	0
	Neuropathy	1	0
	Seizures	6	1
Pulmonary	Pneumonia	2	0
	Infiltrates	1	0
	Dyspnea	4	0
	Acute respiratory distress syndrome	0	1
	Hypoxia	5	2
	Pleural effusion	1	0
	Pulmonary failure	0	3
Renal/genitourinary	Bladder spasms	1	0
	Dysuria	1	0
	Azotemia	11	0
	Renal tubular acidosis	1	0
Secondary malignancy	Epstein-Barr virus lymphoproliferative disorder	0	1
Total		107	20

*Toxicity was assessed by using the modified Common Toxicity Criteria of the National Institutes of Health.

Hepatic complications (34% of patients) were the most frequent organ toxicity; 82% of these were reversible grade III hyperbilirubinemia typically resulting from cholestasis lenta and bilirubin transport dysfunction due to protocol-specified high therapeutic serum levels of CSP within the first month of HCT [13]. No grade IV hepatic events were observed. The 20 grade IV events included cardiac (n = 6), pulmonary (n = 6), neurologic (n = 4), and other (n = 4) toxicities.

Infection Rates in the First 100 Days after HCT

For MMF 3-times-daily patients, the documented rates per 100 patient days of viral (including cytomegalovirus reactivation), fungal, and bacterial infections were 0.86, 0.26, and 1.05 (total infection rate of 2.17), respectively. The rate per 100 patient days of presumed clinical infections was 0.88.

Nineteen (18.5%) of the 103 patients died from nonrelapse causes. The cumulative probability of nonrelapse mortality was 19% at 2 years. Causes of death included grade IV GVHD (n = 3), GVHD complicated by thrombotic thrombocytopenic purpura (n = 1), infections or sepsis syndrome with GVHD (n = 3), infections or sepsis without GVHD (n = 7), aspiration pneumonitis (n = 1), acute respiratory distress after graft failure (n = 1), ventricular dysrhythmia (n = 2), and suspected myocardial infarction (n = 1).

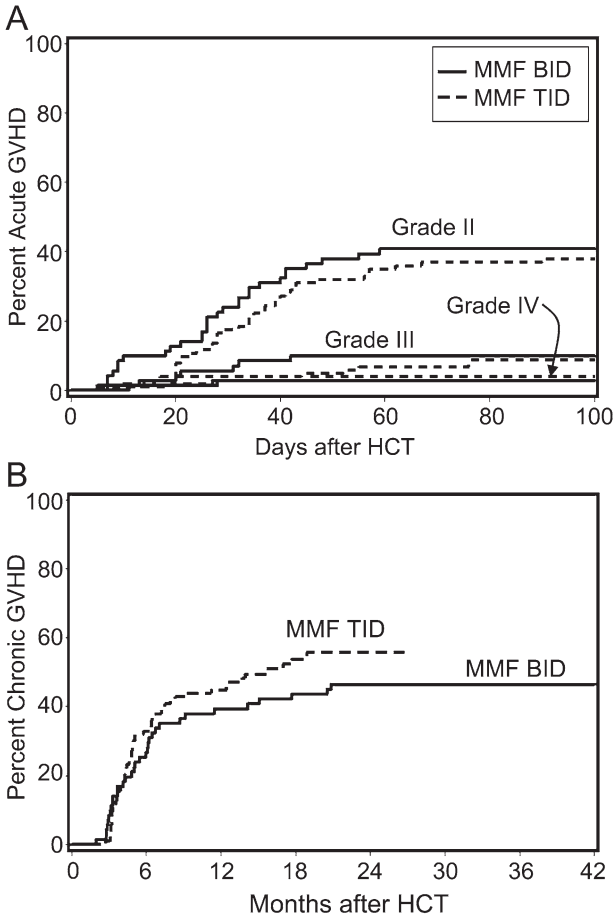

Figure 2. Cumulative probabilities of grades II, III, IV acute GVHD (A) and chronic extensive GVHD (B) between patients given MMF twice (BID) and 3 times (TID) daily.

Graft-versus-Host Disease

The cumulative incidence of grades II to IV acute GVHD was 53% (Figure 2A). Of this, 39% was grade II, 10% was grade III, and 4% was grade IV acute GVHD. Chronic GVHD requiring therapy occurred in 50 patients. The cumulative probability of chronic extensive GVHD at 2 years for all 103 patients was 56% (Figure 2B).

Survival

Sixty-five of 103 patients were alive between 6 and 34.7 months (median, 21.4 months) after HCT. The probability of 2-year survival was 58% (Figure 3). The Kaplan-Meier probability of 2-year survival for patients with lymphoproliferative diseases (NHL and HD, CLL, and Waldenström macroglobulinemia) was 56%; multiple myeloma, 55%; acute leukemias, 68%; CML, 75%; and MDS/MPS, 33%.

Status of Underlying Disease and Progression-Free Survival

Fifty of the 65 surviving patients were in complete remission (CR), 3 were in partial remission, 4 had stable disease, and 8 had relapsed or progressed disease. Nineteen patients (18.5%) died from disease relapse or progression. The Kaplan-Meier estimate of 2-year progression-free survival for the 103 patients was 49% (Figure 3). Disease responses were seen among all disease categories. Among the 61 patients with measurable disease at HCT, the overall response rate was 60%, with 29 (48%) achieving CR, 7 (12%) achieving partial remission, 7 (12%) having stable disease, and 3 not being evaluable because of early nonrelapse deaths. Fifteen (25%) of the 61 patients with measurable disease at HCT had disease progression, including all 4 renal cell carcinoma patients. Two patients who achieved CR eventually relapsed. Eleven (26%) of the 42 patients who were in CR (without measurable disease) at HCT had disease relapse after HCT. The 1- and 2-year cumulative probabilities of relapse/progression were 26% and 31%, respectively. The 2-year Kaplan-Meier estimates of progression-free survivals for patients were as follows: lymphoproliferative diseases (NHL and HD, CLL, and Waldenström macroglobulinemia), 49%; multiple myeloma, 46%; acute leukemias, 66%; CML, 30%; and MDS/MPS, 25%.

Donor Lymphocyte Infusions

Seven of the 103 patients received DLI with a starting dose of 10^7 CD3 cells per kilogram, 5 for progressive disease (HD, n = 2; MDS, n = 2; and renal cell carcinoma, n = 1). The MDS and renal cell carcinoma patients continued to progress. The HD patients received cytoreductive chemotherapy before DLI: 1 had stabilization of disease, whereas the other showed progression. One patient with persistent follicular NHL received a dose of pentostatin 10 mg/m^2 2 days before DLI for low donor T-cell chimerism (35%) at both day 28 and day 44. The patient developed progressively increasing T-cell chimerism and

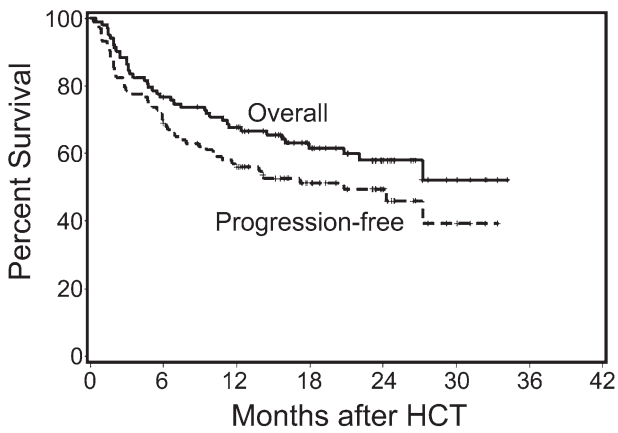

Figure 3. Kaplan-Meier product estimates of overall and progression-free survivals for 103 patients given unrelated donor HCT and MMF 3 times daily.

Table 3. *Comparison of Grade 3 and 4 Organ Toxicities during the First 100 Days between Patients Who Received MMF Twice Daily and Three Times Daily*

Organ System	No. Grade III-IV Toxicities* Twice Daily (n = 71)	3 Times Daily (n = 103)	*P* Value
Cardiovascular	21 (30%)	18 (17%)	.06
Endocrine	0 (0%)	1 (1%)	.30
Gastrointestinal	5 (7%)	10 (10%)	.53
Hemorrhage	5 (7%)	7 (7%)	.95
Hepatic	13 (18%)	34 (33%)	.03
Metabolic	0 (0%)	3 (3%)	.07
Neurologic	8 (11%)	11 (11%)	.90
Pulmonary	14 (20%)	18 (17%)	.71
Renal/genitourinary	3 (4%)	11 (11%)	.11
Second malignancy	0 (0%)	1 (1%)	.30
Any grade III/IV	37 (52%)	60 (58%)	.42

*Toxicity was assessed by using the modified Common Toxicity Criteria of the National Institutes of Health.

experienced a CR but has required therapy for GVHD and infections. The final patient had CR of the underlying CLL but developed Epstein-Barr virus lymphoproliferative disease in donor cells, which was rapidly progressive and only transiently responded to multiagent chemotherapy. After DLI, the patient achieved a CR, as assessed by positron emission and computed tomography scans, but has required treatment for GVHD and infections.

HLA Class I Allele-Level Mismatching

Of the 10 patients with HLA class I allele mismatches, 7 had bidirectional mismatches in both the GVHD and rejection vectors, and 3 had unidirectional mismatches in the rejection vector. One of the 10 patients with single HLA-C allele–mismatched grafts in the rejection vector for CML-CP rejected the graft. The distribution of grades II, III, and IV acute GVHD among the patients who had mismatched grafts was 50%, 10%, and 10%, respectively.

Comparison of Results in Current Patients Given MMF 3 Times Daily (n = 103) with Those in Previous Patients Given MMF Twice Daily (n = 71)

Nonhematopoietic toxicities in the first 100 days after HCT. No significant differences in overall grade III or IV nonhematopoietic toxicities were found (Table 3). Patients given MMF 3 times daily had more hepatic and metabolic toxicities but fewer cardiac grade III or IV toxicities.

Hematopoietic toxicities and transfusions in the first 100 days after HCT. No statistical differences in the degrees or durations of thrombocytopenias or neutropenias were seen. However, the neutrophil nadir was lower for patients given MMF 3 times daily compared with twice daily (100 versus 200 cells per microliter, respectively; $P = .05$). Median numbers of platelet (2 [range, 0-48] versus 0 [range, 0-84], respectively; $P = .91$) and RBC (6 [range, 0-32] versus 7 [range, 0-40 days], respectively; $P = .66$) transfusions in the first 100 days after HCT were not significantly different.

Infectious complications in the first 100 days after HCT. Patients who received MMF 3 times daily had increased rates of documented infections ($P = .004$) attributable to higher viral (including cytomegalovirus reactivation; $P = .01$) and fungal ($P = .02$), but not bacterial ($P = .36$), infections (Table 4). MMF 3-times-daily patients also had higher rates of presumed clinical infections ($P < .0001$) for which pathogenic organisms were not isolated. This remained significant even after correction for patient age and disease risk.

Graft rejection. Patients given MMF 3 times daily experienced fewer rejections (5/103 [5%] versus 13/71 [18%]; $P = .004$; Figure 4). This was true for 3-times-daily patients both with ($P = .08$) and without ($P = .05$) preceding chemotherapy. Multivariate analysis of the combined data from both patient groups showed significantly increased risks of graft rejection for patients with CML (hazard ratio, 9.09; confidence interval, 3.0-27) and MDS/MPS (hazard ratio, 3.45; confidence interval, 1.0-11) compared with all other diagnoses ($P = .0006$; Figure 4). In this model, MMF 3 times daily resulted in a trend toward lessened graft rejection ($P = .11$).

Correlation of day 28 donor T-cell chimerism levels and outcomes. Patients given MMF 3 times daily had a statistically significantly higher median level of donor T-cell chimerism at day 28 (90% versus 75%, respectively; $P = .02$). An analysis of combined data from all

Table 4. *Infection Rates per 100 Days after HCT for Patients Receiving MMF Twice Daily or Three Times Daily*

Variable	Unadjusted MMF Twice Daily†	MMF 3 Times Daily†	Relative Risk	*P* Value	Adjusted* Relative Risk	*P* Value
Documented	1.5	2.1	1.4	.004	1.4	.01
Viral	0.53	0.86	1.6	.01	1.6	.03
Fungal	0.11	0.26	2.4	.02	2.4	.04
Bacterial	0.91	1.05	1.2	.36	1.1	.48
Clinical	0.15	0.88	5.8	<.0001	6.4	<.0001

*Adjusted for patient age and disease risk.
†Infection rates per 100 days.

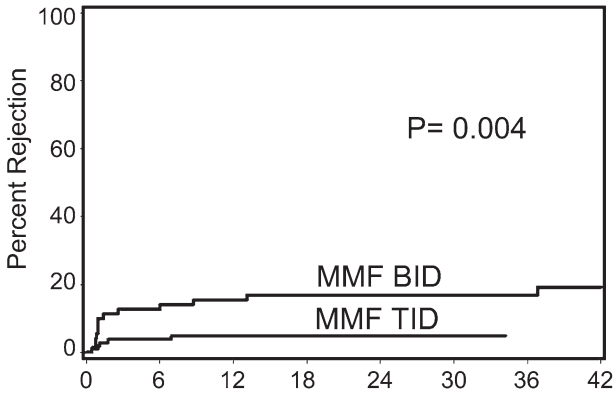

Figure 4. Cumulative probability estimates of rejection for 174 G-PBMC recipients given MMF twice (BID) or 3 times (TID) daily.

174 patients showed that patients with ≤50% donor T-cell chimerism had (1) a greater risk of eventual graft rejection ($P \leq .0001$); (2) lower risks of acute and chronic GVHD ($P = .02$ and .0002, respectively); (3) a higher risk of relapse ($P = .05$); and (4) a trend toward worse progression-free survival ($P = .11$). No correlations between donor T-cell chimerism levels and survival ($P = .78$) or nonrelapse mortality ($P = .95$) were found.

Acute and chronic GVHD. There were no differences in the times of onset and the cumulative incidences of grades II to IV ($P = .37$) or III to IV ($P = .91$) acute GVHD (Figure 2A). Also, both times of onset and cumulative incidences ($P = .46$; Figure 2B) of chronic extensive GVHD were similar.

We next examined risk factors for acute and chronic GVHD among all 174 G-PBMC recipients. Having >2 prior chemotherapy regimens ($P = .04$) was associated with a higher risk of grades III or IV acute GVHD. Both numbers of prior chemotherapies and greater patient age were strongly associated with greater risks of chronic GVHD necessitating therapy ($P = .0002$ and .005, respectively). To determine whether reduction of host lymphocytes by chemotherapy before HCT could explain the higher incidence of acute GVHD among patients with >3 compared to ≤3 prior chemotherapy regimens, lymphocyte counts between day −7 and day −4 were averaged and found to be 790 and 1060 /μL, respectively ($P = .04$).

Nonrelapse mortality, relapse/progression, and progression-free survival. No differences in nonrelapse mortality were seen ($P = .79$). There was a trend toward less relapse/progression for patients given MMF 3 times daily ($P = .08$).

DISCUSSION

The postgrafting immunosuppression with MMF and CSP in the current nonablative HCT protocol has been aimed not only at controlling GVHD, but also, as clearly shown in preclinical canine studies [2], at facilitating allogeneic engraftment. The previously published study of unrelated HCT used twice-daily dosing of MMF [1]. The 18% incidence of graft rejection in G-PBMC recipients, coupled with an unexpected short half-life of only 3 hours of the active MMF metabolite, mycophenolic acid (MPA), suggested that increased MMF dosing by using a 3-times-daily schedule might reduce the incidence of graft rejection and, moreover, reduce acute GVHD. How successful has this intervention been? The sustained engraftment rate for G-PBMC recipients increased from 82% to 95% ($P = .004$). Specifically, durable engraftment for patients with and without chemotherapy increased from 90% to 97% and from 47% to 83%, respectively. The predetermined end point of achieving a rejection rate of <10% was statistically demonstrated ($P = .05$) for patients with prior chemotherapy, but for those without such therapy, the end point of <20% was not achieved with sufficient statistical power in this small patient subgroup. We believe that the data are strong enough to advocate that patients given unrelated donor HCT after FLU/2-Gy TBI conditioning should receive 3-times-daily MMF. However, patients without preceding chemotherapy, in particular CML patients, will require, in addition, more intensive pretransplantation immunosuppressive conditioning to achieve acceptable rates (>90%) of donor engraftment [14-16], and a TBI dose-escalation protocol has been initiated to determine the level of immunosuppression required.

We evaluated plasma concentrations of MPA, the active metabolite of MMF, and outcomes in 38 patients of the previous study given MMF 15 mg/kg twice daily and 47 patients of the current study given MMF 15 mg/kg 3 times daily. MPA pharmacokinetics were determined on days 7 and 21. Comparing the twice-daily and 3-times-daily MMF group, the mean total MPA concentration steady states (Css) were 1.9 and 3.1 μg/mL and the unbound Css were 18 and 36 ng/mL, respectively ($P < .0001$) [17]. Sixteen of the patients who had total MPA Css <3 μg/mL had low (<50%) donor T-cell chimerism ($P = .03$), and 6 of the patients with MPA Css <2.5 μg/mL had graft rejection. We concluded that 3-times-daily dosing of MMF led to increased MPA Css, which, in turn, predicted higher degrees of donor T-cell chimerism. Given the strong association between high levels of donor T-cell chimerism and sustained engraftment [18], targeting MPA Css >2.5 μg/mL could prevent graft rejection.

In contrast to the apparent benefit on engraftment, MMF 3-times-daily dosing did not improve acute GVHD; the overall and individual grade II, III, and IV incidences of acute GVHD were similar to those seen on the original MMF twice-daily protocol.

The reasons for the lack of effect on GVHD were not clear. Nevertheless, the relatively low rates of grade III or IV acute GVHD seen with both twice-daily and 3 times a day MMF dosing (10% and 12%, respectively) compared favorably to the 30% to 47% rates observed after conventional unrelated donor HCT [19,20]. We have recently reported that there was no beneficial relationship between acute GVHD (as opposed to chronic GVHD) and GVT responses after nonmyeloablative conditioning, but grade III or IV acute GVHD significantly increased nonrelapse mortality [7]. Therefore, future trials will focus on further reducing the incidence of acute GVHD after unrelated nonablative HCT.

Besides increased infections in patients given MMF, the total incidences of grade III or IV organ toxicities were similar in both patient groups. Fortunately, the higher infection rates did not translate into increased nonrelapse mortality. Perhaps the increase in infections was due to maintaining full-dose MMF 3-times-daily patients who developed GVHD with consequent greater impairment of host immune responses. Gastrointestinal and hematologic toxicities were not increased in patients given MMF 3 times daily except for slightly lower neutrophil nadirs. The reasons for the differences in grade III or IV cardiovascular and hepatic toxicities between protocols were not clear but could have been related to differences in pre-HCT organ comorbidities [19,21].

Given that acute and chronic GVHD were not reduced and that infections increased in MMF 3-times-daily patients, the duration of 3-times-daily MMF dosing might be shortened from 96 to 28 days. Durable engraftment was highly correlated with the presence of ≥50% donor T-cell chimerism levels at day 28 [18,22], and 3-times-daily dosing could safely be converted to twice-daily dosing in patients with ≥50% donor T-cell levels at 4 weeks.

This study showed a tumor response rate of 60% (48% CR) in patients with measurable disease at the time of HCT, and the cumulative probability of relapse at 2 years was 31% in these patients, similar to the 27% rate in patients who were in CR before HCT. These rates were better than those seen in previous patients given MMF twice daily [1] and argue that greater immunosuppression with MMF 3 times daily did not blunt GVT effects. Overall, this translated into trends toward decreased relapse/progression and better overall and progression-free survivals for patients treated with MMF 3 times daily. In part, the improved tumor control might have been the result of the higher observed donor chimerism levels [18] and better sustained engraftment rates. In part, the nature of the underlying diseases might have played a role, because patients with myeloid malignancies, such as MDS, MPS, and CML, who had the highest risks of relapse, were more commonly represented on the MMF twice-daily protocol.

The overall analyses of data from the combined cohort of all 174 G-PBMC recipients confirmed earlier observations of the effects of high levels (>50%) of day 28 donor T-cell chimerism on sustained engraftment, on acute and chronic GVHD, and on disease relapse [1,18]. Also, more heavily pretreated patients had a moderately increased risk for grade III or IV acute GVHD and had a substantially higher risk for chronic extensive GVHD. Older patient age was independently associated with a higher risk for chronic extensive GVHD. Age has been a well-described risk factor for acute and chronic GVHD after conventional HCT in patients with hematologic malignancies [23] and for chronic, but not acute, GVHD in patients who undergo transplantation for aplastic anemia [24,25]. A higher number of prior chemotherapy regimens possibly reflected lower numbers of intact host immune-regulatory cells, and this was crudely shown by lower lymphocyte counts in patients who received >3 chemotherapy regimens compared with those with less chemotherapy. The lack of intact host regulatory T-cell responses has been thought to result in more rapid donor T-cell engraftment and homeostatic proliferation [26,27], thus resulting in reduced tolerance induction [28].

The results of this study seemed comparable to those of others that used more intensive reduced-intensity conditioning regimens, as shown in Table 5. Most studies were small, including <50 patients. Despite more intensive pretransplantation conditioning and, in some studies, the addition of in vivo T-cell depletion with antithymocyte globulin or alemtuzumab, the graft rejection rates ranged from 4.5% to 23%, which was not better than the results with the current nonmyeloablative regimen. Similar to our study, higher-than-expected graft rejection rates (23%) were identified for CML patients given high-dose-rate single exposure of 550-cGy TBI combined with FLU. The rates of grade II to IV acute and chronic GVHD in these studies have ranged from 6% to 60% and 0% to 59%, respectively, and have generally been lower after in vivo alemtuzumab. The current nonrelapse mortality rate (19%) was at the lower end of rates reported in these studies (range, 19.8%-55%). The overall and progression-free survivals at 2 years of 58% and 49%, respectively, for current patients given MMF 3 times daily seemed comparable to the corresponding rates of 44% to 76% and 37% to 62%, respectively, identified at 1 to 2 years in these other studies.

In summary, FLU 90 mg/m^2 and 2-Gy TBI conditioning followed by postgrafting immunosuppression with CSP twice daily and MMF 3 times daily was highly effective for achieving unrelated donor G-PBMC engraftment in patients with malignancies except for

Table 5. *Outcomes of Reduced-Intensity Unrelated Donor HCT for Hematologic Malignancies*

Study	Diagnosis (n)	Conditioning	Postgrafting IS	Graft Failure	Grades II-IV Acute/Chronic GVHD	NRM	OS/DFS
Girgis [29]	Heme. Malig. (110)	550 cGy TBI/Cy	CSP/MTX	7.7%	30%/59%	19% (low risk); 42% (high risk)	47%/40%
Hallemeier [16]	CML (22)	550 cGy TBI/Cy	CSP/MTX/Pred	23%	–	–	–
Bornhauser [30]	Heme. Malig. (44)	IV Bu/FLU/ATG	CSP/MTX or CSP/MMF	21%	32%/21%	25%	DFS 64%, 38%, and 14% (lymphoid, standard, and high-risk leukemia, respectively) at 13 mo
Chakraverty [31]	Heme. Malig. (47)	Mel/FLU/alemtuzamab	CSP	4.5%	6%/0%	19.8	76%/62% at 2 y
Wong [32]	Myeloid (29)	Mel/FLU or IV Bu/FLU/ATG	CSP/MTX	14%	41%/50%	55%	44%/37% at 1 y
Shimoni [33]	Heme. Malig. (36)	Mel/FLU or IV Bu/FLU: ± ATG or alemtuzamab	CSP/MTX	11%	31%/45%	39%	52%/43% at 1 y
Rodriguez [34]	Heme. Malig. (22)	Mel/FLU	CSP/MMF	4.5%	63%/52%	32%	59%/55% at 1 y

ATG indicates antithymocyte globulin; Bu, busulfan; CML, chronic myeloid leukemia; CSP, cyclosporine; Cy, cyclophosphamide; DFS, disease-free mortality; FLU, fludarabine; Heme. Malig, hematologic malignancy; IS, immunosuppression; IV, intravenous; Mel, melphalan; MTX, methotrexate; NRM, nonrelapse mortality; OS, overall survival; Pred, prednisone; TBI, total body irradiation.

those with CML, and this has now become the standard approach for unrelated HCT after this particular nonmyeloablative regimen. For CML patients, a TBI dose-escalation study will determine the minimal dose of TBI necessary for reliable engraftment. Although rates of grades III and IV acute GVHD were relatively low, a further reduction in GVHD and associated morbidity and mortality would be desirable before extending the application of this regimen to patients with nonmalignant disorders of hematopoiesis.

ACKNOWLEDGMENTS

Supported in part by grant nos. CA78902, K23 CA92058, CA18029, CA15704, CA49605, HL36444, and DK56465 from the National Institutes of Health, Department of Health and Human Services, Bethesda, MD. The authors thank Paul Martin for helpful discussions regarding GVHD and the data coordinators Heather Hildebrandt and Debbie Bassuk and the study nurses Steve Minor, Mary Hinds, and John Sedgwick for their invaluable help in making the study possible. The authors also thank Helen Crawford, Bonnie Larson, and Sue Carbonneau for manuscript preparation; all physicians, nurses, and support personnel for their care of patients on this study; and the patients for their willingness to participate in these clinical trials.

REFERENCES

1. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood*. 2003;102:2021-2030.
2. Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood*. 1997;89:3048-3054.
3. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood*. 1998;92:3515-3520.
4. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295-304.
5. Thomas ED, Storb R, Clift RA, et al. Bone-marrow transplantation. *N Engl J Med*. 1975;292:832-843, 895-902.
6. Sullivan KM. Graft-versus-host disease. In: Thomas ED, Blume KG, Forman SJ, eds. *Hematopoietic Cell Transplantation*. Malden, MA: Blackwell Sciences, Inc.; 1999:515-536.
7. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol*. 2005;23:1993-2003.
8. Bethge WA, Storer BE, Maris MB, et al. Relapse or progression after hematopoietic cell transplantation using nonmyeloablative conditioning: effect of interventions on outcome. *Exp Hematol*. 2003;31:974-980.

9. Bethge WA, Hegenbart U, Stuart MJ, et al. Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Blood*. 2004;103:790-795.
10. Sandmaier BM, Maloney DG, Maris MB, et al. Conversion of low donor chimerism following nonmyeloablative conditioning for hematopoietic cell transplantation (HCT) using pentostatin and donor lymphocyte infusion (DLI) [abstract 186]. *Blood*. 2004;104(pt 1):57a.
11. Durnam DM, Anders KR, Fisher L, O'Quigley JO, Bryant EM, Thomas ED. Analysis of the origin of marrow cells in bone marrow transplant recipients using a Y-chromosome-specific in situ hybridization assay. *Blood*. 1989;74:2220-2226.
12. Ugozzoli L, Yam P, Petz LD, et al. Amplification by the polymerase chain reaction of hypervariable regions of the human genome for evaluation of chimerism after bone marrow transplantation. *Blood*. 1991;77:1607-1615.
13. Hogan WJ, Maris M, Storer B, et al. Hepatic injury after nonmyeloablative conditioning followed by allogeneic hematopoietic cell transplantation: a study of 193 patients. *Blood*. 2004; 103:78-84.
14. Baron F, Maris MB, Storer BE, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with chronic myeloid leukemia. *Biol Blood Marrow Transplant*. 2005;11:272-279.
15. Hallemeier C, Girgis M, Blum W, et al. Outcomes of adults with acute myelogenous leukemia in remission given 550 cGy of single-exposure total body irradiation, cyclophosphamide, and unrelated donor bone marrow transplants. *Biol Blood Marrow Transplant*. 2004;10:310-319.
16. Hallemeier C. Re: High rate of graft failure in 25 patients with chronic myelogenous leukemia conditioned with a reduced-intensity regimen of 550 cGy total body irradiation and cyclophosphamide for unrelated donor transplantation [letter to the editor]. *Biol Blood Marrow Transplant*. 2004;10: 726-727.
17. Giaccone L, McCune JS, Maris MB, et al. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. *Blood*. 2005;106:4381-4388.
18. Baron F, Baker JE, Storb R, et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *Blood*. 2004;104:2254-2262.
19. Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplant comorbidities. *Blood*. 2004;104:961-968.
20. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood*. 2003;102:756-762.
21. Sorror M, Maris M, Baron F, et al. A modified hematopoietic cell transplantation (HCT)-specific co-morbidity index [abstract 1146]. *Blood*. 2004;104(pt 1):324a-325a.
22. Baron F, Storb R, Gooley T, et al. Assessing donor chimerism level among CD3 T, CD4 T, CD8 T, and NK cells predicts subsequent graft rejection, GVHD, and relapse after allogeneic HCT with nonmyeloablative conditioning [abstract 31]. *Biol Blood Marrow Transplant*. 2005;11(suppl):11.
23. Weisdorf D, Hakke R, Blazar B, et al. Risk factors for acute graft-versus-host disease in histocompatible donor bone marrow transplantation. *Transplantation*. 1991;51:1197-1203.
24. Storb R, Prentice RL, Sullivan KM, et al. Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. *Ann Intern Med*. 1983;98:461-466.
25. Storb R, Prentice RL, Buckner CD, et al. Graft-versus-host disease and survival in patients with aplastic anemia treated by marrow grafts from HLA-identical siblings. Beneficial effect of a protective environment. *N Engl J Med*. 1983;308:302-307.
26. Ernst B, Lee D-S, Chang JM, Sprent J, Surh CD. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity*. 1999;11:173-181.
27. Goldrath AW, Bevan MJ. Low-affinity ligands for the TCR drive proliferation of mature CD8+ T cells in lymphopenic hosts. *Immunity*. 1999;11:183-190.
28. Wu Z, Bensinger SJ, Zhang J, et al. Homeostatic proliferation is a barrier to transplantation tolerance. *Nat Med*. 2004; 10:87-92.
29. Girgis M, Hallemeier C, Blum W, et al. Chimerism and clinical outcomes of 110 unrelated donor bone marrow transplants who underwent conditioning with low-dose, single-exposure total body irradiation and cyclophosphamide. *Blood*. 2005;105:3035-3041.
30. Bornhauser M, Thiede C, Platzbecker U, et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res*. 2001;7:2254-2262.
31. Chakraverty R, Peggs K, Chopra R, et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood*. 2002;99:1071-1078.
32. Wong R, Giralt SA, Martin T, et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. *Blood*. 2003;102:3052-3059.
33. Shimoni A, Kröger N, Zabelina T, et al. Hematopoietic stem-cell transplantation from unrelated donors in elderly patients (age >55 years) with hematologic malignancies: older age is no longer a contraindication when using reduced intensity conditioning. *Leukemia*. 2005;19:7-12.
34. Rodriguez R, Parker P, Nademanee A, et al. Cyclosporine and mycophenolate mofetil prophylaxis with fludarabine and melphalan conditioning for unrelated donor transplantation: a prospective study of 22 patients with hematologic malignancies. *Bone Marrow Transplant*. 2004;33:1123-1129.